*American Journal of Experimental Agriculture*
*3(1): 1-13, 2013*

**SCIENCEDOMAIN** ***international***
*www.sciencedomain.org*

# Exogenous Application of Glycinebetaine Facilitates Maize (*Zea mays* L.) Growth under Water Deficit Conditions

**K. Raja Reddy[1*], W. Brien Henry[1], Ramdeo Seepaul[1], Suresh Lokhande[1], Bandara Gajanayake[1] and David Brand[1]**

*[1]Department of Plant and Soil Sciences, Mississippi State University, Box 9555, Mississippi State, MS 39762, USA.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author KRR designed, wrote the protocol, and executed the study. All authors participated in data collection. Authors SL, BG and DB managed the analysis of the study. Author RS wrote the first draft. All authors read and approved the final manuscript.*

***Research Article***

***Received 6th July 2012***
***Accepted 12th November 2012***
***Published 2nd December 2012***

## ABSTRACT

**Aims:** To determine whether the exogenous application of glycinebetaine (GB) can ameliorate the effects of water deficit on maize growth and physiological processes.
**Study Design:** Split plot design with water deficit being the main plot factor and GB application being the subplot factor. Treatment was a combination of water deficit level and GB application with 3 replications.
**Place and Duration of Study:** R.R. Foil Plant Science Research Center, Mississippi State University, Mississippi State, MS, USA between May and July 2010.
**Methodology:** A pot experiment was conducted using 31-d old 'TV25R19' maize irrigated with 750 ml pot$^{-1}$ day$^{-1}$ (WW: well-watered), 450 mL pot$^{-1}$day$^{-1}$ (WD60, 60% of WW) and 300 mL pot$^{-1}$day$^{-1}$ (WD40, 40% of WW) grown with or without GB application at each stress level. GB was applied as a foliar spray every 5 days at a rate of 4 kg ha$^{-1}$. Soil moisture content and leaf water potential, growth, biomass, and gas exchange parameters were measured in response to the treatment variables.
**Results:** Significant GB and water deficit main effects were observed for plant height (PH), leaf dry weight (LDW), ear dry weight (EDW) and total dry weight (TDW) ($P \leq 0.05$) while

---

**Corresponding author: Email: krreddy@pss.msstate.edu;*

GB main effects alone were observed for node number (NN) and stem dry weight (SDW) ($P \leq 0.05$). GB application increased leaf area (LA) (5,454 $cm^2$ $plant^{-1}$) in WD60 plants relative to untreated plants. No GB effect was seen under other treatment combinations at 10 or 20 days after treatment (DAT) measurements. GB did not increase stomatal conductance or transpiration at 10 or 20 DAT in plants subjected to water deficit. GB application resulted in leaf water potential values in the WD60 treatment that were statistically similar to the well-watered plants. Volumetric soil water content did not change with foliar GB application across water deficit treatments except under mild stress after 18 DAT, where soil moisture was higher for GB treated plants.

**Conclusion:** GB's effect was most evident in plants from the WD60 treatment. GB application significantly improved PH, LA, LDW, SDW, EDW and TDW and did not influence NN under WD60 conditions.

*Keywords: Maize; glycinebetaine; water deficits; growth; development; photosynthesis; pigments.*

## 1. INTRODUCTION

Maximizing yield potential involves optimization of farm management practices. Conservation tillage, mulching, irrigation methods, timing and frequency are useful strategies to abate water deficit and stabilize crop yield. In addition to modifying cultural practices, exogenous application of compatible solutes such as proline [1], glycinebetaine (GB) [2-3] and salicylic acid [4] have improved the drought tolerance of field crops in some years and environments but the results are often inconclusive.

GB is an organic compatible solute that accumulates in plants subjected to water deficit. GB is a metabolically inert amino acid derivative, which acts as an osmoregulant. GB is endogenously synthesized in response to water deficit in the following plant families: *Amaranthaceae, Asteraceae, Capparaceae, Chenopodiaceae, Convulvulaceae, Malvaceae, Poaceae* and *Portulacaceae* [3, 5-6]. In addition to its osmoregulatory role in bacteria and plants [7-8], GB stabilizes cell structures and enzyme activities, protects functional proteins, and maintains the integrity of cell membranes against different stressors [9-12]. GB is a small, highly water soluble molecule that is uniformly neutral with respect to enzyme functions in the cytoplasm, even if present at high concentrations. GB facilitates the maintenance of the water potential equilibrium in the cell which in turn, maintains the turgor pressure during water deficit conditions.

Exogenous application of GB has been reported to enhances water stress tolerance in barley (*Hordeum vulgare*) [13], sorghum (*Sorghum bicolor*) [3], sunflower (*Helianthus annuus*) [4], common beans (*Phaseolus vulgaris*) [2], and soybean (*Glycine max*) [14]. However, GB was not effective in wheat (*Triticum aestivum*) [3]. The effects of GB on plants vary in response to crop, cultivar, rate and application timing and environmental/location effects. Maize has the capacity to absorb and accumulate high levels of exogenous foliar applied GB [15] with shoot to root translocation beginning almost immediately after application [16]. GB is made of small, electrically neutral molecules which are non toxic even at high concentrations [17]. GB was found to be predominantly phloem-mobile, and is partly translocated with assimilates to actively growing shoots and developing organs [16]. GB was also reported to be highly stable in plant tissues remaining unmetabolized up to 17 days after application [16].

Maize cultivars differ in their capacity to synthesize GB [18-19] and this may diminish some low-GB-producing cultivar's ability to function under low moisture availability. Photosynthesis depends on the application rate of exogenous GB with low concentrations (2-20 m*M*) enhancing photosynthesis and growth and higher concentrations (>20 m*M)* decreasing growth and photosynthesis [15]. Lowered stomatal conductance associated with high GB application resulted in reduced photosynthetic capacity. In a field study, Agboma et al. [3] reported that 6 kg $ha^{-1}$ exogenously applied GB increased maize grain yield from 3.2 to 4.3 and 4.2 to 5.0 Mg $ha^{-1}$ under well-watered and deficit conditions, respectively.

Extensive literature is available about the effects on water deficit on maize growth and physiology, however, the use of GB to mitigate the effects of water deficit on growth and physiological processes is inadequately studied. Therefore, the objective of this study was to determine GB effects on maize yield parameters and physiology under three water deficit regimes.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Conditions

This experiment was conducted during the 2010 growing season at the R.R. Foil Plant Science Research Center, Mississippi State University, Mississippi State, MS, USA (33º 28'N, 88º 47'W). Growing season precipitation (May-July) totaled 199 mm and was 30% lower than the long-term average precipitation (260 mm). Within the same period, mean air temperature was 25.7ºC and was close to the long-term average temperature (24.9°C). A locally adapted cultivar 'TV25R19' (Terral Seed Company, Lake Providence, LA, USA) was seeded on 17 May 2010 in 12-L, free draining polyvinyl chloride pots (66 cm depth x 15 cm diameter) each filled with fine sand providing a growing column with volume 11,700 $cm^3$. Nutrients and water were supplied through a drip irrigation system. The experimental design was a split plot with water deficit being the main plot factor and GB application being the subplot factor. Treatment was a combination of water deficit level and GB application with 3 replications consisting of 20 pots per replication. Eleven rows of 40 pots were set side by side in 7-m long rows consisting of two groups of 20 pots each. Each group of 20 pots was spaced 1 m apart. All rows were arranged in an east-west direction with 1-m spacing between rows. Three rows were dedicated to each water deficit level; a single border row in the same configuration was used on each side of the experiment. The west half of each row (20 pots) was sprayed with GB solution with the remaining 20 pots per row sprayed with water only. Seedlings were thinned to one per pot 7 days after emergence.

### 2.2 Irrigation Treatments and GB Application

All pots were well-watered with full-strength Hoagland nutrient solution from emergence to 31 days after seeding (DAS). Pots were covered with plastic sheeting at the base of the plants to prevent rainwater getting into the pots. Three irrigation treatments, WW: 100% irrigation (free drainage from pot), WD60: 60% irrigation of WW, and WD40: 40% irrigation of WW were imposed at 31 DAS similar to Kakani et al [20]. Water deficit treatments were achieved by decreasing the irrigation duration relative to WW treatment. Irrigation duration for WW, WD60 and WD40 was 240, 144, and 96 s, respectively applied at 0800, 1200, and 1600 h through pressure compensated drippers. Each dripper delivered 70 mL of water and nutrients per 60 s. The daily total water and nutrient delivered per pot to WW, WD60 and WD40 treatments were 0.84, 0.504 and 0.336 L, respectively. GB (Nutristim, Min. 97 %

betaine anhydrous, Finnfeeds Finland Ltd., Finland) solution was mixed at a rate of 85.6 g granular powder to 3 L of water and applied 31, 36, 41, 46, 51 and 56 DAS over the top of plants using a $CO_2$ pressurized backpack sprayer at a rate of 4 kg $ha^{-1}$ with 0.1% Tween 20 surfactant and calibrated to spray 140.3 L $ha^{-1}$.

## 2.3 Soil and Leaf Water Content

Midday leaf water potential (LWP) and daily soil moisture content (SMC) were measured at 5-day interval. The top most fully expanded leaves were detached to determine the LWP using pressure chamber (Model 3000, Soil Moisture Equipment Corp., Santa Barbara, CA). SMC of the upper 10-15 cm of soil for each treatment was measured with soil moisture sensors (Type 5-TM, Decagon Devices, Inc. WA, USA). This data were logged every 15 minutes by Campbell scientific unit with CR-1000 data logger (Campbell Scientific, North Logan, UT, USA) from 0 to 25 days after imposing treatments.

## 2.4 Growth, Biomass, and Photosynthesis Measurements

Plant height and node numbers were measured from soil level to the collar of the last unfolded leaf from 0 to 20 days after treatment (DAT) at 5-day interval. At 30 DAT, all plants were harvested to determine leaf, stem, and plant biomass. Photosynthesis parameters such as leaf photosynthesis, stomatal conductance, and transpiration rates were measured at 10 and 20 DAT using the Li-COR 6400 Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA) with an integrated fluorescence chamber. Measurements were made with the following leaf chamber conditions: 1,500 µmol photon $m^{-2}$ $s^{-1}$ photosynthetically active radiation, 30ºC cuvette temperature, and 360-ppm $CO_2$ concentration. Measurements were made using the topmost fully expanded leaf, from three plants per replicate, between 10:30 and 13:00 h on mostly sunny days.

## 2.5 Pigments

Total chlorophyll and carotenoid concentrations were measured from the topmost fully expanded leaves at 10 and 20 DAT. The pigments were extracted by placing 5, 0.38 $cm^2$, leaf disks in a vial containing 5 ml of dimethyl sulfoxide and incubating them in darkness for 24 h. Thereafter, the absorbance of the supernatant was measured at 648, 662, and 470 nm with a Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The total chlorophyll and carotenoids were estimated and expressed on a leaf area basis (µg $cm^{-2}$) using an equation developed by Lichtenthaler [21].

## 2.6 Statistical Analysis

Plant growth and biomass data were subjected to analysis of variance using PROC GLM procedure in SAS (SAS Institute Inc, 2004). The photosynthetic parameters and chlorophyll data were analyzed using repeated measure mixed model analysis of variance, PROC MIXED procedure in SAS (SAS Institute Inc, 2004) for treatment and DAT interactions.

## 3. RESULTS AND DISCUSSION

### 3.1 Growth Characteristics

There was no GB×water deficit interaction in relation to all growth related characteristics measured in this study. However, significant GB and water deficit main effects were observed for plant height (PH), leaf dry weight (LDW), ear dry weight (EDW) and total dry weight (TDW) ($P = 0.05$) while GB main effects alone were observed for node number (NN) and stem dry weight (SDW) ($P = 0.05$). In general, measured growth characteristics declined linearly with increased water deficit intensity, both with and without GB application (Table 1). Plant height declined with water deficit and was affected by GB application only under WD60 conditions, exhibiting a 14% (21.4 cm) increase in height with GB application (Table 1) over untreated plants. Average node number of well-watered plants (18.0) differed from WD60 and WD40 plants (16.6), and no GB effect was observed on node numbers (Table 1). Similar to plant height, leaf area and total dry weight (along with its components, leaf and stem dry weights) declined with increased water deficit and were unaffected by GB except under WD60 conditions. Plant height is strongly correlated to node numbers; however, in our study GB application increased plant height without influencing the node numbers under WD60 (WW and WD40 were unaffected by GB application) stress conditions. This may be due to increased internodal lengths enhanced by the GB application, an undocumented effect. Leaf area averaged 6,066 cm$^2$ plant$^{-1}$ and 3,048 cm$^2$ plant$^{-1}$ under well-watered and WD40 conditions, respectively, unaffected by GB application. However, under WD60 conditions, GB application increased leaf area (5,454 vs. 3,620 cm$^2$ plant$^{-1}$) with GB results undifferentiated from well-watered plants' leaf area (Table 1). LA increased with GB application (Table 1) under mild stress even though the node numbers did not differ, suggesting that leaf length and width increased with GB application resulting in similar LA to well-watered plants' LA. GB, therefore, ameliorated the effect of water deficit on growth characteristics of maize under mild stress conditions. The positive effects of GB application in maize can be linked to its physiological role as an osmoprotectant that enhances drought tolerance [3].

**Table 1. The influence of glycinebetaine and water deficit on maize growth, yield and yield components**

| Treatment | Plant height (cm) | Node number | Leaf area (cm$^2$ pl$^{-1}$) | Leaf dry weight | Stem dry weight | Ear dry weight | Total dry weight |
|---|---|---|---|---|---|---|---|
| | | | | g pl$^{-1}$ | | | |
| WW | 205.3$^a$ | 18.0$^a$ | 6129.1$^a$ | 47.5$^a$ | 123.1$^a$ | 39.64$^b$ | 210.2$^a$ |
| WW + GB | 208.0$^a$ | 18.0$^a$ | 6002.9$^a$ | 48.6$^a$ | 119.2$^{ab}$ | 47.08$^a$ | 214.9$^a$ |
| WD60 | 155.3$^c$ | 16.3$^b$ | 3620.0$^b$ | 35.8$^b$ | 75.8$^c$ | 17.22$^d$ | 128.8$^c$ |
| WD60 + GB | 176.7$^b$ | 17.0$^b$ | 5453.7$^a$ | 46.4$^a$ | 101.2$^b$ | 26.59$^c$ | 174.1$^b$ |
| WD40 | 136.3$^d$ | 16.7$^b$ | 2943.3$^b$ | 30.0$^b$ | 67.5$^c$ | 10.15$^e$ | 107.6$^c$ |
| WD40 + GB | 141.3$^{dc}$ | 16.3$^b$ | 3153.5$^b$ | 33.8$^b$ | 75.3$^c$ | 13.87$^{ed}$ | 122.9$^c$ |
| LSD (5%) | 14.7 | 0.7 | 1081.0 | 7.9 | 21.4 | 5.6 | 31.2 |

*Note: GB=Glycinebetaine, WW=well-watered, WD60=60% of WW and WD40=40% of WW*
**Within columns, mean followed by the same letter are not significantly different at 0.05 level of probability*

Total dry weight followed similar trends, with well-watered plants yielding an average of 213 g plant$^{-1}$ while WD40 plants yielding an average of 115 g plant$^{-1}$. Once again, under WD60 conditions, GB application increased TDW from 129 to 174 g plant$^{-1}$, a 35% increase. Leaf and stem dry weight followed trends similar to TDW. Ear dry weight also decreased with water deficit severity. GB increased ear dry weight in both well-watered and WD60 conditions by 15 and 35%, respectively. GB treated plants under WD40 conditions did not differ in ear dry weight from untreated plants.

In our study, water deficit reduced TDW and its components. This study suggests that exogenous GB application may help to mitigate the effects of water deficit in maize under mild stress conditions (WD60). GB's influence on these measured characteristics was not detectable under well-watered (WW) and severe stress conditions (WD40). Because the compound is highly stable, benefits may be accrued later in the season, beyond the termination date of this experiment, once GB is accumulated to effective threshold levels which may offset the decrease in growth and gas exchange processes caused by severe water deficit. In this study, the influence of GB on total dry weight under mild stress conditions results from an increase in plant height and its strong correlation with leaf area, leaf, stem and ear dry weight.

## 3.2 Gas Exchange Processes

The effect of exogenous GB on gas exchange processes under water deficit conditions was studied to determine the effect of GB on ameliorating stress effects on these physiological processes. Water deficit conditions can limit photosynthesis considerably through stomatal or biochemical limitations. To determine the effect of GB on gas exchange processes, photosynthesis measurements taken 10 DAT and 20 DAT were analyzed using mixed model with repeated measures to determine the DAT×GB and DAT×water deficit effects. Photosynthesis varied with DAT×GB interaction ($P \leq 0.05$) which arose from 24% lower photosynthesis with GB application under WD60 conditions at 20 DAT. Compared to 10 DAT, photosynthesis was lower for all treatment combinations at 20 DAT except untreated WD60 plants (Table 2). No GB effect was observed under other treatment combinations at either 10 or 20 DAT (Table 2). Averaging the two DAT measurements, photosynthesis linearly decreased with (slope = -5.7, $r^2$ = 0.94) and without (slope = -5.3, $r^2$ = 0.97) GB application with increasing water deficit.

GB did not increase stomatal conductance or transpiration at 10 and 20 DAT in plants subjected to water deficit (Table 2). At 20 DAT, stomatal conductance was higher in well-watered GB treated plants by 76%; however, stomatal conductance was lower in GB plants under WD60 (Table 2). Internal $CO_2$ concentration was also lowered with increasing water deficit severity at 10 and 20 DAT and was not influenced by GB application. Stomatal conductance and internal $CO_2$ concentration were strongly correlated with photosynthesis for GB treated and untreated plants.

GB application protects the photosynthetic machinery when exposed to water deficit conditions in bean (*Phaseolus vulgaris*) [2] and maize [3]. Yang and Lu [15] found that photosynthesis increases with GB concentration in the range of 2-20 m*M*, above which photosynthetic capacity is reduced in maize. This increase can be related to PSII photochemistry in particular the photochemical quenching coefficient ($q_P$) which facilitates greater electron transport in PSII [15]. In addition, the ameliorating effect of GB on photosynthesis can be attributed to increased stomatal conductance augmented by increased turgor pressure in guard cells. In our study, water deficit lowered photosynthesis, and GB application did not ameliorate the effect of water deficit on maize photosynthetic capacity. Similarly, stomatal conductance and transpiration decreased with increasing water deficit.

**Table 2. The influence of glycinebetaine and water deficit on maize gas exchange processes: net photosynthetic rate, stomatal conductance and transpiration rate and internal $CO_2$ at 10 and 20 days after treatment (DAT)**

| **Treatment** | **Net photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)** | | | **Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)** | | | **Transpiration ($mmol\ m^{-2}\ s^{-1}$)** | | | **Internal $CO_2$ ($C_i$) (ppm)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **10 DAT** | **20 DAT** | ***p*-value** | **10 DAT** | **20 DAT** | ***p*-value** | **10 DAT** | **20 DAT** | ***p*-value** | **10 DAT** | **20 DAT** | ***p*-value** |
| WW | $39.67^{ab}$ | $33.43^{a}$ | .02 | $0.69^{a}$ | $0.26^{c}$ | < .001 | $10.75^{ab}$ | $10.87^{ab}$ | .94 | $265.6^{a}$ | $242.3^{ab}$ | .08 |
| WW + GB | $40.77^{a}$ | $36.07^{a}$ | .06 | $0.53^{ab}$ | $0.58^{a}$ | .06 | $12.06^{a}$ | $11.65^{ab}$ | .79 | $235.0^{ab}$ | $275.6^{a}$ | .29 |
| WD60 | $34.43^{bcd}$ | $32.80^{a}$ | .48 | $0.51^{ab}$ | $0.39^{b}$ | .23 | $10.06^{ab}$ | $14.40^{a}$ | .01 | $248.3^{a}$ | $176.3^{c}$ | < .001 |
| WD60 + GB | $36.87^{abc}$ | $25.03^{b}$ | < .001 | $0.57^{ab}$ | $0.20^{cd}$ | < .001 | $11.23^{ab}$ | $10.69^{ab}$ | .72 | $260.6^{a}$ | $227.6^{b}$ | .15 |
| WD40 | $28.67^{d}$ | $23.37^{b}$ | .03 | $0.27^{c}$ | $0.13^{d}$ | .16 | $7.66^{c}$ | $8.27^{b}$ | .63 | $188.6^{b}$ | $136.8^{c}$ | .03 |
| WD40 + GB | $31.47^{cd}$ | $22.50^{b}$ | < .001 | $0.37^{bc}$ | $0.13^{d}$ | .02 | $8.96^{bc}$ | $8.33^{b}$ | .68 | $215.3^{ab}$ | $163.6^{cd}$ | .03 |
| LSD (5%) | 5.96 | 3.68 | | 0.24 | 0.11 | | 2.34 | 4.15 | | 55.8 | 37.3 | |

*Note: GB=Glycinebetaine, WW=well-watered, WD60=60% of WW and WD40=40% of WW. Within columns, means followed by the same letter are not significantly different at 0.05 level of probability P value to compare days after treatment (DAT) within gas exchange processes*

In addition, GB did not influence stomatal conductance and transpiration in plants subjected to water deficit contrary to the findings of Ma et al. [22] and Mäkelä et al. [23]. Photosynthesis was strongly correlated with stomatal conductance and intercellular $CO_2$ concentration (*C*i) for both GB treated and untreated plants suggesting that photosynthesis in maize leaves may be subjected to both stomatal and biochemical limitations. In our study, photosynthesis was strongly correlated with plant height, node numbers, leaf area and TDW in GB treated plants.

## 3.3 Pigment Concentrations

Generally, total chlorophyll and total carotenoids concentrations were not reduced as water deficit increased, with or without GB application at 10 DAT and 20 DAT (Table 3). However, under WD60 conditions, GB application caused a significant decrease in total chlorophyll at 10 DAT. This effect was not observed at 20 DAT. Total chlorophyll and carotenoids were consistently lower at 20 DAT for most treatment combinations (Table 3). Total chlorophyll for GB treated well-watered and WD60 plants were similar across the two measurement dates.

**Table 3. The influence of glycinebetaine and water deficit on total chlorophyll and carotenoids content in maize at 10 and 20 days after treatment (DAT)**

| **Treatment** | **Total chlorophyll** µg cm$^{-2}$ | | ***P*-value** | **Carotenoids** µg cm$^{-2}$ | | ***P*-value** |
|---|---|---|---|---|---|---|
| | 10 DAT | 20 DAT | | 10 DAT | 20 DAT | |
| WW | $45.9^a$ | $43.4^a$ | .07 | $8.1^{ab}$ | $5.2^{ab}$ | < .001 |
| WW + GB | $44.2^a$ | $40.5^{ab}$ | .01 | $8.6^{ab}$ | $6.1^a$ | < .001 |
| WD60 | $43.4^a$ | $38.5^{bc}$ | < .001 | $8.1^{ab}$ | $5.8^a$ | < .001 |
| WD60 + GB | $39.5^b$ | $40.5^{ab}$ | .44 | $8.9^a$ | $5.7^a$ | < .001 |
| WD40 | $44.9^a$ | $38.6^{bc}$ | < .001 | $7.6^b$ | $5.9^a$ | < .001 |
| WD40 + GB | $43.7^a$ | $36.4^c$ | < .001 | $8.5^{ab}$ | $3.7^b$ | < .001 |
| LSD (5%) | 3.5 | 3.0 | | 1.3 | 1.7 | |

*Note: GB=Glycinebetaine, WW=well-watered, WD60=60% of WW, WD40=40% of WW Within columns, mean followed by the same letter are not significantly different at 0.05 level of probability. P value to compare days after treatment with pigments*

## 3.4 Leaf and Soil Water Status

Leaf water potential decreased with increasing water deficit severity. However, no leaf water potential difference was observed between GB treated and untreated well-watered and WD60 plants ranging from an average of -1.24 MPa under well-watered conditions to -1.59 MPa under WD60 conditions (Fig. 1). Under WD40, GB treated plants (-1.77 MPa), leaf water potential was less negative than untreated plants (-1.85 MPa) ($P \leq 0.05$).

GB did not offset the effects of water deficit on LWP for well-watered and mild stress conditions. This finding is contrary to Xing and Rajashekar [2] who found that the LWP of GB treated plants declined at a lower rate than untreated bean plants as water deficit increased. In this study, average (over sampling dates) LWP for severely stressed plants for the duration of the study revealed that GB treated plants (-1.77 MPa) were less negative than untreated plants (-1.85 MPa) suggesting that GB application may delay the onset of water deficit effects on maize growth and physiological processes under WD40 growing conditions. Volumetric soil water content was not affected by GB application in the well-watered and WD40 treatments, but in WD60 at 18 DAT, soil moisture was higher for GB treated plants (Fig. 2). Soil water status is affected by evapotranspiration processes, therefore any plant growth factor such as rooting depth, plant height, leaf area or stomatal conductance will influence soil water status. We were interested in determining whether GB influence on growth and physiological processes relate to the soil water status. Similar to LWP, volumetric soil water content did not change with foliar GB application across water deficit treatments. However, under mild water deficit, we found that there is a divergence between GB treated and untreated soil water status from 18 DAT, whereby soil moisture was higher for GB treated plants.

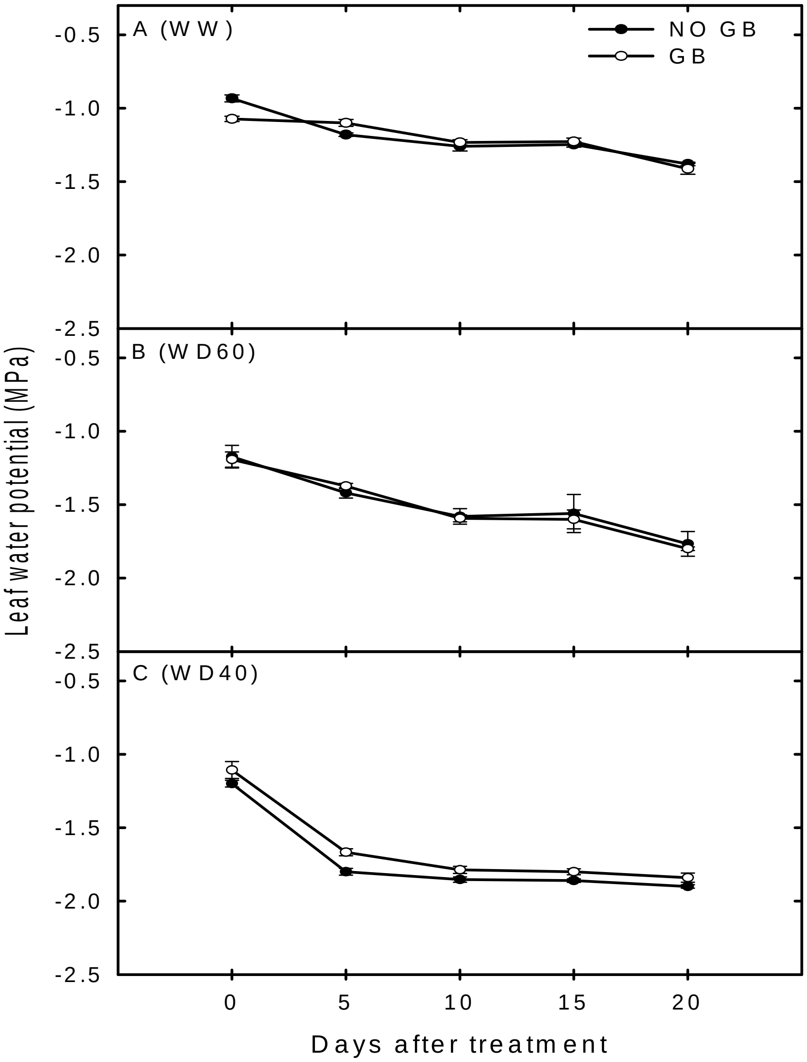

**Fig. 1. Temporal changes in midday leaf water potential of maize grown in well-watered (WW), mild (WD60) and severe water deficit (WD40) plants treated with (GB) and without (No GB) foliar glycinebetaine (GB) application**

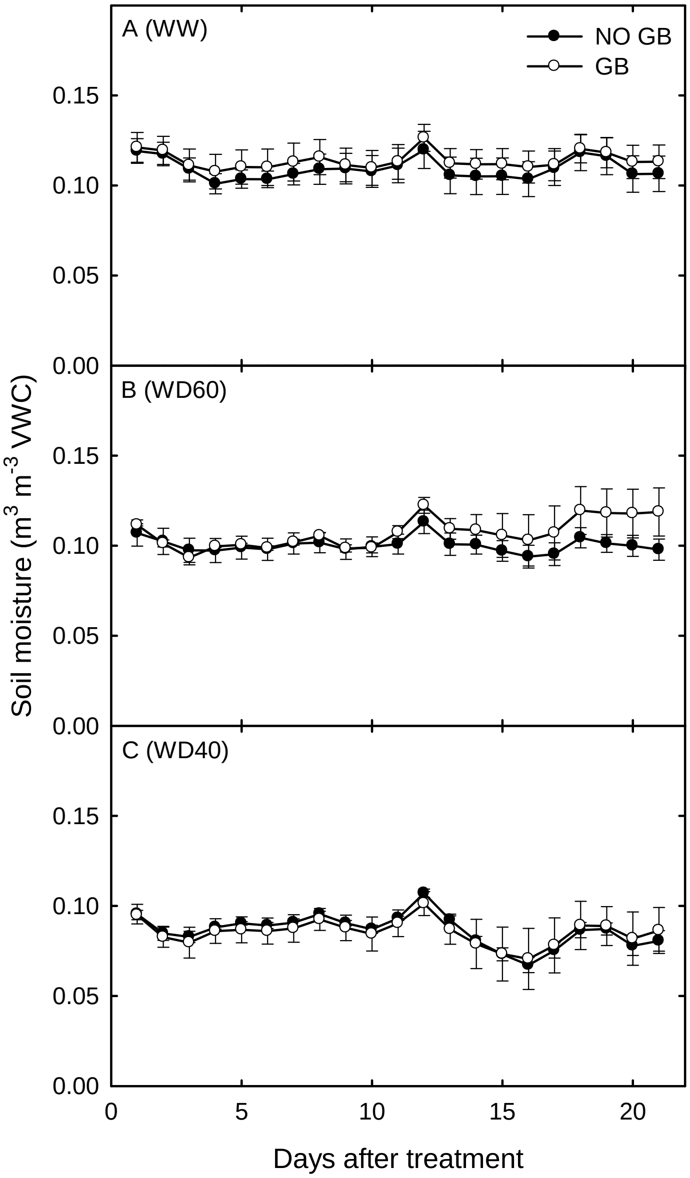

**Fig. 2. Temporal changes in soil moisture content (m3 m-3) of maize grown in well-watered (WW), mild (WD60) and severe water deficit (WD40) plants treated with (GB) and without (No GB) foliar glycinebetaine (GB) application**

This difference in soil water status at 18 DAT may be related to the activation of GB as an osmoprotectant which may have stabilized membranes by maintaining PSII protein conformation, osmotic adjustments maintaining turgor pressure and scavenging reactive oxygen species under water deficit conditions (Reddy et al., 2004), likely resulting in reduced transpiration rates. GB treated plants that were under mild stress resulted in increased growth, TDW and its components (Table 1) while the soil moisture content was higher >18 DAT (Fig. 2), GB therefore may have contributed to the plant avoiding the water deficit by increasing the canopy transpiration efficiency of mildly stressed maize. Alternatively, GB may simply slow down transpiration and discourage injury to the leaf, in a mechanism that is not documented. GB accumulation at active levels, 60 DAT, may have allowed plants to function under low moisture availability. In addition, GB treated plants under mild stress conditions had higher specific leaf area (117.54 cm2 g-1) than untreated plants (101.12 cm2 g-1), suggesting that the GB application caused thinner leaves perhaps by inducing fewer mesophyll cells per unit area which, in turn, caused by to cell expansion under rapid growth. Turgor pressure is critical in gas exchange processes by moderating stomatal conductance and photosynthesis [24]. GB may be involved in modulating turgor pressure [25] which is the driving force in plant cell expansion, hence the observed increase in biomass rendered by GB application observed.

### 3.5 Leaf Area Profiling

Leaf area (LA) profiling showed a difference among leaves 5, 10 and 15 for all treatment conditions. Generally leaf area increased from leaf 5 to 10 subsequently declining from leaf 10 to 15. Leaf 5 area was inconsistent across treatments (Fig. 3). In contrast, for plants treated with GB, area of leaves 10 and 15 increased under well-watered (25 to 27%) and WD40 (14 to 15%).

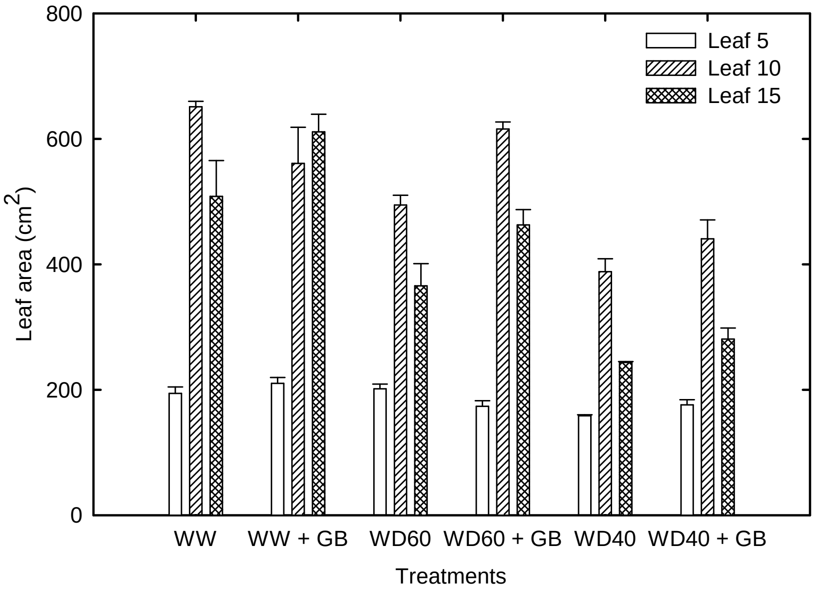

**Fig. 3. Leaf area of 5th, 10th and 15th leaf of maize grown in well-watered (WW), mild (WD60) and severe water deficit plants (WD40) treated with (GB) and without (No GB) foliar glycinebetaine (GB) application**

## 4. CONCLUSION

In conclusion, exogenous application of GB was only advantageous under mild water deficit conditions. Photosynthesis was found to linearly decrease with and without GB application under water deficit conditions suggesting GB did not influence photosynthesis. Stomatal conductance was lower while internal $CO_2$ concentration increased in GB treated plants exposed to mild water deficit relative to untreated plants. The effect of water deficit on plant height and biomass and its components were offset by GB application under mild stress conditions. GB application allowed plants in the mildly-stressed treatment to overcome water limitation and continue growing which resulted in increased biomass relative to the untreated mildly stressed plants.

## ACKNOWLEDGEMENTS

Part of this research was supported by the Mississippi Corn Promotion Board and Danisco Animal Nutrition Company for the product. This is a contribution from the Department of Plant and Soil Sciences, Mississippi State University and Mississippi Agricultural and Forestry Experiment Station, no. J-12138.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Heuer B. Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. Plant Sci. 2003;165(4):693-9.
2. Xing W, Rajashekar CB. Alleviation of water stress in beans by exogenous glycine betaine. Plant Sci. 1999;148(2):185-92.
3. Agboma PC, Jones MGK, Peltonen Sainio P, Rita H, Pehu E. Exogenous glycinebetaine enhances grain yield of maize, sorghum and wheat grown under two supplementary watering regimes. J Agron Crop Sci. 1997;178(1):29-37.
4. Hussain M, Malik MA, Farooq M, Ashraf MY, Cheema MA. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. J Agron Crop Sci. 2008;194(3):193-9.
5. Wyn Jones RG, Gorham J. Aspects of salt and drought tolerance in higher plants. In: Kosuge T, Meredith C, Hollaender A, editors. Genetic Engineering of Plants: An Agricultural Perspective Plenum Press, New York; 1983, p. 355-70.
6. McNeil SD, Nuccio ML, Hanson AD. Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol. 1999;120(4):945-9.
7. Smith LT, Smith GM, Madkour MA. Osmoregulation in *Agrobacterium tumefaciens*: accumulation of a novel disaccharide is controlled by osmotic strength and glycine betaine. J Bacteriol. 1990;172(12):6849-55.
8. [8] Grumet R, Hanson AD. Genetic evidence for an osmoregulatory function of glycinebetaine accumulation in barley. Funct Plant Biol. 1986;13(3):353-64.
9. Nawaz K, Ashraf M. Exogenous application of glycinebetaine modulates activities of antioxidants in maize plants subjected to salt stress. J Agron Crop Sci. 2010;196(1):28-37.
10. Incharoensakdi A, Takabe T, Akazawa T. Effect of betaine on enzyme activity and subunit interaction of ribulose-1, 5-bisphosphate carboxylase/oxygenase from *Aphanothece halophytica*. Plant Physiol. 1986;81(4):1044-9.
11. Papageorgiou GC, Fujimura Y, Murata N. Protection of the oxygen-evolving photosystem II complex by glycinebetaine. Biochim Biophys Acta Bioenergetics. 1991;1057(3):361-6.

12. Zhao Y, Aspinall D, Paleg LG. Protection of membrane integrity in *Medicago sativa* L. by glycinebetaine against the effects of freezing. J Plant Physiol. 1992;140(5):541-3.
13. Mäkelä P, Mantila J, Hinkkanen R, Pehu E, Peltonen-Sainio P. Effect of foliar applications of glycinebetaine on stress tolerance, growth, and yield of spring cereals and summer turnip rape in Finland. J Agron Crop Sci. 1996;176(4):223-34.
14. Agboma PC, Sinclair TR, Jokinen K, Peltonen-Sainio P, Pehu E. An evaluation of the effect of exogenous glycinebetaine on the growth and yield of soybean: timing of application, watering regimes and cultivars. Field Crops Res. 1997;54(1):51-64.
15. Yang X, Lu C. Effects of exogenous glycinebetaine on growth, $CO_2$ assimilation, and photosystem II photochemistry of maize plants. Physiol Plant. 2006;127(4):593-602.
16. Mäkelä P, Peltonen-Sainio P, Jokinen K, Pehu E, Setala H, Hinkkanen R, et al. Uptake and translocation of foliar-applied glycinebetaine in crop plants. Plant Sci. 1996;121(2):221-30.
17. Reddy AR, Chaitanya KV, Vivekanandan M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol. 2004;161(11):1189-202.
18. Rhodes D, Rich PJ. Preliminary genetic studies of the phenotype of betaine deficiency in *Zea mays* L. Plant Physiol. 1988;88(1):102-8.
19. Brunk DG, Rich PJ, Rhodes D. Genotypic variation for glycinebetaine among public inbreds of maize. Plant Physiol. 1989;91(3):1122-5.
20. Kakani VG, Reddy KR, Zhao D. Deriving a simple spectral reflectance ratio to determine cotton leaf water potential. J New Seed. 2007;8(3):11-27.
21. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol. 1987;148:350-82.
22. Ma XL, Wang YJ, Xie SL, Wang C, Wang W. Glycinebetaine application ameliorates negative effects of drought stress in tobacco. Russian J Plant Physiol. 2007;54(4):472-9.
23. Mäkelä P, Kontturi M, Pehu E, Somersalo S. Photosynthetic response of drought- and salt-stressed tomato and turnip rape plants to foliar-applied glycinebetaine. Physiol Plant. 1999;105(1):45-50.
24. Ludlow MM, Fisher MJ, Wilson JR. Stomatal adjustment to water deficits in three tropical grasses and a tropical legume grown in controlled conditions and in the field. Funct Plant Biol. 1985;12(2):131-49.
25. Lv S, Yang A, Zhang K, Wang L, Zhang J. Increase of glycinebetaine synthesis improves drought tolerance in cotton. Mol Breed. 2007;20(3):233-48.

---

*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/abstract.php?iid=156&id=2&aid=708.*